

**Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol**

**Supplemental Assay Method for Sterility Testing of
Preparations Other Than Live Vaccines**

Date: December 16, 1998

Supersedes: New

Number: STSAM0906.01

Standard Requirement: 9 CFR 113.26

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1. Introduction

This Supplemental Assay Method describes the test procedure used to detect viable bacteria and fungi in all biological products other than live vaccines, per the Code of Federal Regulations, Title 9 (9 CFR), Part 113.26. In the presence of these contaminating extraneous agents, the medium will be rendered turbid by macroscopic examination.

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

- 2.1.1 Individually packaged sterile 3-cc syringes
- 2.1.2 Vacutainer needles
- 2.1.3 30°-35°C incubator
- 2.1.4 20°-25°C incubator
- 2.1.5 Bunsen burner
- 2.1.6 Biosafety cabinet
- 2.1.7 Individually packaged sterile 1-cc pipets

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

- 2.2.1 Soybean casein digest medium (SCDM) or trypticase soy broth (TSB), fluid thioglycollate medium (FTM), and/or fluid thioglycollate medium with beef (FTMw/Bf). See **Appendix 8.1** - Media Formulations.
- 2.2.2 Glassware: tubes and flasks with media
- 2.2.3 Sterile water in serum vials: volumes determined by products to be tested

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2.2.4 Sterile clothes: coveralls, mask, hair bonnet, sleeves, shoe covers, gloves, and protective eyewear

2.2.5 70% ethyl alcohol

2.2.6 0.05% Germ Warfare disinfectant

2.2.7 4 x 4 sterile gauze pads

2.2.8 Clean-Pal wipes

3. Preparation for the test

3.1 Personnel qualifications/training

3.1.1 The executor must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The executor must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent; and training in the operation of the necessary laboratory equipment listed in **part 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn biosafety cabinets on at the beginning of the work week and leave on all week.

3.2.2 Monitor incubators daily for temperature according to the current version of NVSLSOP0001.

3.2.3 Monitor freezers and coolers for storage of biologicals for temperature daily according to the current version of NVSLSOP0003.

3.3 Preparation of reagents/control procedures

3.3.1 Positive controls: Use *Bacillus subtilis*, *Candida krusei*, and *Clostridium chauvoei* (or equivalent organisms as specified in the current United States Pharmacopoeia (USP) as the positive controls in order to determine the growth promoting qualities of the medium according to 9 CFR 113.25 (b). Conduct these

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positive control tests on each autoclave lot of media according to the current versions of STSAM0900 and STSAM0901.

3.3.2 Prepare the *B. subtilis*, *C. krusei*, and *C. chauvoei* reagents according to the current version of the STRPP0001 protocol.

3.3.3 Technique Controls: Inoculate each of 20 test vessels of media with 1 ml sterile water from serum vials of the same size as used to rehydrate those biologicals tested. All volumes of water used on the tested biologicals are used in the technique controls. If no water vials were needed with the tested biologicals, inoculate 20 test vessels with 1 ml of water from 10 serum vials containing 5 ml of sterile water each. Use the same boxes or lots of syringes used with the tested serials of biologicals to inoculate the sterile water of the technique controls. Incubate 10 technique control media test vessels at each incubation temperature for the 14 days of the test.

3.3.4 Negative or Media Controls: Incubate 20 representative test vessels of uninoculated media to confirm the sterility of the autoclaved media (9 CFR 113.25 (c)). Incubate 10 representative test vessels at each incubation temperature for the 14 days of the test.

3.4 Preparation of the samples

3.4.1 The biological samples to be tested are received from the Biological Material Processing Section (BMPS), as stated in the current version of STSOP0001.

3.4.2 Check in the biological samples, check the serial numbers of all vials, record the diluent numbers, assign a test number, and complete the testing log book, as stated in the current version of STSOP0010.

3.4.3 Look up the volume of test medias needed for each serial to be tested on the dilution of preservative computer disk using the notebook program and the file name A:\VOLLIST4, as described in the

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current version of STSOP0020. Record volumes used in the log book.

3.4.4 Order sufficient media from the NVSL media section for delivery 1 day before the biological samples are to be tested. Determine the type of media to be ordered by test code for each product (see Appendix 8.2). Order additional media to cover positive controls, negative controls, technique controls, and subcultures.

3.4.5 Order sterile purified water in serum vials from the NVSL media section in sufficient volumes, as stated on the label or in the outline, for those serials without accompanying diluent. Order sterile water for the technique controls also. NOTE: Nearly all nonlive products are liquid; any reference to diluent/rehydration is only occasionally needed.

4. Performance of the test

4.1 On the day of the test, wipe off the serials of biologic to be tested with 0.05% Germ Warfare using a Clean-Pal (or equivalent). Pay special attention to the cleaning of the tops of the vials and rubber stoppers.

4.2 Set the wiped-down samples on a tray and place inside the outer sterility room on a table.

4.3 The executor then gowns up for sterility testing by wearing sterile coveralls, booties, sleeves, mask, hair bonnet, gloves, and protective eyewear.

4.4 Wipe down the interior surfaces of the biosafety cabinet used for testing with 70% alcohol (or equivalent).

4.5 Number the media test vessels to coincide with the serials to be tested.

4.6 Place testing materials (syringes, vacutainer needles, 4 x 4 gauze squares, etc.) in the biosafety cabinet or on a cart next to the cabinet.

4.7 Place samples and test media for the first serial in the biosafety cabinet.

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4.8 Swab the tops of the samples with a 4 x 4 gauze pad soaked in 70% alcohol. Flame the tops of the samples using a Bunsen burner. Equivalent methods for decontaminating the tops of the samples may be used.

4.9 One by one, rehydrate each vial of the serial, if needed, using a syringe and needle or vacutainer needle. Use the firm's diluent if provided and sterile water if not. Consecutively rehydrate 10 vials of each serial. When testing master cell samples, there will occasionally be less than 10 vials. At least 20 ml of master cell sample is needed for testing.

4.10 Inoculate 1 ml from each vial of the liquid, rehydrated liquid, or thawed frozen liquid product into each of 2 test vessels of media. The volume of media in these test vessels was determined in **3.4.3**. If 1-ml vials are tested, only 0.5 ml will be inoculated in each test vessel.

4.11 Repeat **parts 4.7-4.10** on the other serials of biologic to be tested this day. When this day's testing is complete, the executor then initials and dates the log book as "test on."

4.12 Do the technique controls next. Inoculate 20 test vessels with approximately 1.0 ml of sterile water, using syringes and needles of the same lot as were used with the tested serials.

4.13 Place the test vessels in the appropriate incubator temperature. Also place 10 technique control test vessels at each incubator temperature and 10 negative or broth control test vessels at each incubator temperature. The volumes of media used for negative or broth controls should be representative of those volumes used with test serials.

4.14 Clean up the sterility room by wiping down the interior of the biosafety hood and counter tops with 70% alcohol. Remove paper trash from the sterility room and discard the biological samples and any extra media by autoclaving.

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5. Interpretation of the test results

5.1 Examine all test vessels for cloudiness, either due to the product or a contaminant, at least 1 time during days 7-11 of the incubation period. If it is not possible to tell if the cloudiness is due to a contaminant, subculture the serial. To subculture, place 1 ml from the test vessel into 40 ml of fresh test media using a sterile individually packaged 1-ml pipet. If less than 10 test vessels of a serial are cloudy, subculture those that are, or at least 3 vessels. If all 10 test vessels are cloudy, randomly pick 3 test vessels at each incubation temperature to be subcultured.

5.2 At the end of the test, on day 14, examine all test vessels and subculture tubes for macroscopic growth. Do a microscope slide from all tubes which appear to have macroscopic growth. After these slides have dried, Gram stain and observe them with a microscope. Record the number of tubes with growth and no-growth, as well as the results of any Gram stains, in the log book for this test code.

5.3 If extraneous growth is observed in any test vessel and confirmed by Gram stain, conduct 1 retest using 20 unopened final container samples. Record the results of any retest in the log book for this test code.

5.4 If no extraneous growth is found in any test vessel of the initial test or any vessel of the retest, the serial is satisfactory (SAT).

5.5 If extraneous growth is found in any test vessel of the retest, the serial of biologic is unsatisfactory (UNSAT).

5.6 If a serial is found unsatisfactory, freeze down 3-4 ml of contaminated media in the central Revco and label the tube with the test code, the serial's test number, and the date. Also save the Gram-stained slide.

6. Report of test results

6.1 Record test results indicating the number of vessels with no growth over the number tested and the test conclusion of SAT or UNSAT in the testing log book and on the computer test sheet for each serial tested. The person taking the test off on the 14th day should initial and date the log book and computer test sheet.

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6.2 Enter the results recorded on the test sheet in the computer. A computer printout of the result is received and checked against the computer test sheet and log book for accuracy by the person entering the results. See the current version of STSOP0021 for directions on entering test results.

6.3 Give the test result printouts and log book to the Sterility/Cytology microbiologist or supervisor to check, sign, and date.

6.4 The person doing the test and paperwork then validates the test results in the computer (STSOP0021).

6.5 File the signed and validated test report printouts in the SEA files under the first 2 numbers of each serial's product code. File the BMPS test sheet by test code in the same file drawer.

7. References

7.1 Code of Federal Regulations, Title 9, Parts 113.25 & 113.26, U.S. Government Printing Office, Washington, DC, 1996.

7.2 The U.S. Pharmacopoeia, 1985, Vol. 21, pp. 1151-1160, Mack Publishing Co., Easton, PA.

8. Appendices

8.1 Media formulations

8.1.1 NVSL Media Formulation No. 10423

**TRYPTICASE SOY BROTH (TSB)
or
SOYBEAN CASEIN DIGEST MEDIUM (SCDM)**

Trypticase Soy Broth	30 g
QH ₂ O	1000 ml

Autoclave 20 min at 121°C.
TSB and SCDM are 2 names for the same media formulation
from different media companies.

8.1.2 NVSL Media Formulation No. 10135

FLUID THIOGLYCOLLATE MEDIUM (BBL)

Fluid Thioglycollate Medium	29.5 g
QH ₂ O	1000 ml

Mix and heat to boiling.
Autoclave 20 min at 121°C.

8.1.3 NVSL Media Formulation No. 10227

FLUID THIOGLYCOLLATE WITH BEEF EXTRACT

Fluid Thioglycollate Medium	29.5 g
QH ₂ O	1000 ml

Heat and add:
0.5% Beef Extract (DIFCO) 5 g

Bring to a boil and dispense.
Autoclave 20 min at 121°C.

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8.2 Media to be used with each test code

026-ST0-----FTM-30°-35°C SCDM-20°-25°C	Killed products without merthiolate or clostridial components
026-ST1-----FTM-30°-35°C FTM-20°-25°C	Killed products with merthiolate but no clostridial components
026-ST2-----FTMw/Bf-30°-35°C SCDM-20°-25°C	Killed products with clostridial components but no merthiolate
026-ST3-----FTMw/Bf-30°-35°C FTM-20°-25°C	Killed products with clostridial components and merthiolate
026-ST4-----FTM-30°-35°C SCDM-20°-25°C	Plasma bags without merthiolate
026-ST5-----FTM-30°-35°C FTM-20°-25°C	Plasma bags with merthiolate